# Nanotechnology improves disease resistance in plants for food security: Applications and challenges

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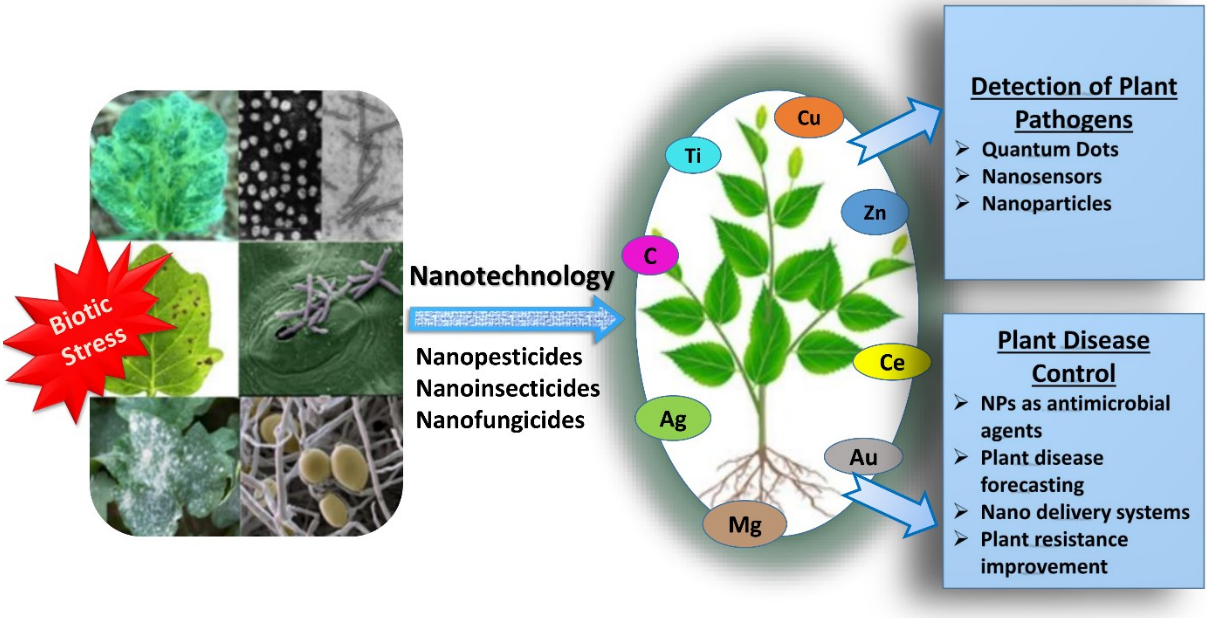
## Abstract

Green synthesis of nano-fertilizers is emerging as a potential strategy and could play a crucial role in disease mitigation, diagnosis, or suppression. Different nanoscale devices (nanoparticles, NPs), biosensors, nano-diagnostic kits, nanofabrication, nanobarcodes, microRNA detection, quantum dots, and nanopore sequencing systems can be used to diagnose plant biotic stress. New research innovations include nanoformulations (nanogels, nanosuspensions, nanoemulsions) and various types of nanoparticles that are useful as nanopesticides (e.g., nanoinsecticids, nanobactericides, nanofungicides and nanonematicides) to enhance plant productivity. These nanomaterials may be involved in different mechanisms of pathogen interactions with plants e.g., ROS production, expression of stress-resistant genes, pathogen cell lysis, and DNA mutation. The optimum use of nano-fertilizers and nanopesticides is a remedy for agriculture and the food industry. The present study endeavors to unveil the mechanisms behind developing resistance against new biotic stresses in fruits and vegetables, and therefore to develop exciting new techniques to resist biotic stress.

**Key Words**: Biotic stress, defense mechanisms, phytopathogens, phytonanotechnology, resistance, stress mitigation

## Graphical Abstract



## 1. Introduction

In agriculture, biotic stresses such as pathogen infection, herbivore attack, and pest and insect incidence are major challenges to protecting food from spoilage. As a physiological, genetic, and ecological unit, plants survive in the presence of various microorganisms that can be pathogenic, neutral, or beneficial. Pathogenic microorganisms, pests, insects, and herbivores are the causes of biotic stresses that negatively impact the health and function of plants (Kumari et al., 2021). Pests damage around 40% of the global crop and phytopathogenic organisms, as averred by Food and Agriculture Organization (FAO) officials of the United Nations(Balaure et al., 2017 ; Mitra. 2021). Pest and infected crop losses are the greatest menace to food security and the farming community across the globe (Pandey et al., 2017). Some international agricultural agencies have reported crop damage caused by biotic stresses exceeds fifty percent of the total crop yield in the world (Pestovsky et al., 2017). Moreover, plants' carbohydrate metabolism and yield are affected when pathogens interact with them (Berger et al., 2007). If seen economically, cash crops get damaged due to biotic stressors, causing monetary loss (Thind. 2012). The yearly crop loss is worth 2000 billion dollars worldwide caused by the biotic stresses of plants (Oluwaseun and Sarin. 2017).

Along with the crop losses, the bulging human population poses a major problem. In this grave situation, a major task is to satisfy the needs of increasing numbers withrestricted resources. The state of global food security is alarming because it is hard to maintain the balance between the growing population and growing food demand (Savary et al., 2012). The situation is worsening because of rapidly changing climate conditions, which have increased the incidence of biotic stresses. Plants’ disease management is primarily responsible for these concerning conditions (Younas et al., 2020). Typically, chemicals such as fungicides, bactericides, and nematicides are used extensively to control different pathogens, but pollute the environment and affect the ecosystems as well as human health (Sarkar et al., 2022). Therefore, conventional techniques are not sustainable and are not cost-effective due to their inherent expense and time consumption (Tanwar and Sushil. 2019).

Most importantly, 90% of the chemicals used in controlling biotic diseases escape into the environment during application. Often the crop only needs a minute amount of the applied chemical fertilizers; therefore, their extensive use has polluted both soils and water (Kaningini et al., 2022). In addition, the continued use of pesticides and fungicides accrues the danger of toxic chemicals accumulating in food products, affecting the food chain and compromising both plant and human health. Many pesticides applied to crops are carcinogenic; moreover, they are resistant to decomposition, which is alarming for health security (Tanwar and Sushil. 2019). The conventional approaches to mitigate biotic stress are not promising. Therefore, agricultural systems must incorporate sustainable modern technologies to deal with biotic stresses and increase crop production (Kumari et al., 2021). In this regard, nanotechnology is a possible elixir to ensure food security and maintain plant health. Nanotechnology is the science, design, production, engineering, application, and characterization of nanoparticles for the benefit of the human being (Krishna et al., 2017). This technology is multidisciplinary as well as interdisciplinary, which deals with particles having a size of 0.1-100 nm. Nanotechnology can outperform more conventional approaches in crop production. Nanomaterials improve soil health, seed germination, crop growth, gene expression, ecological sustainability, and plant stress tolerance (Banerjee and Kole. 2016 ; Khan et al., 2021). Phytonanotechnology is used to give plants resistance to mitigate biotic stress. Nanosensors/nanobiosensors, nanopesticides, nanofungicides, and nanofertilizers can be used for disease diagnostics and control, increase crop yield, and effective crop management after harvest (Kerry et al., 2017). Nanotechnology can also manage biotic stress or strengthen the plant's immune system against disease (Younas et al., 2020).

Nanoparticles (NPs), because of their small size, easy transportation, high efficiency, and long shelf life, are the most favorable choice for agriculturists over other classical methods. In addition, the efficiency of nanoparticles favors their widespread application in plant pathology and provides eco-friendly and effective management options for biotic stress. In this way, nanotechnology can aid agriculture and alleviate ecological challenges through the sustainable use of nanopesticides, nanofungicides, and nanobactericides against plant diseases (Hazarika et al., 2022). Various nanomaterials (NMs) such as nano-chitosan, nano-silver, nano-silica, and nano-copper help manage plant disease. Moreover, less water-soluble fungicides are provided with organic solvent in the form of nanoparticles. Nanoparticles function as carriers of fungicides to improve low water solubility, enhance stability and decrease volatilization (Worrall et al., 2018). The larger surface area owing to the minuscule size and high activity of nanoparticles, is their greatest advantage: this greater efficiency can be widely used in human and plant pathology (Marwal et al., 2018). Hence, in the agriculture sector, the next phase of excellence in precision farming methods, genetically engineered crops, and chemical pesticides will likely be facilitated and framed by ongoing research and development in nanotechnology.

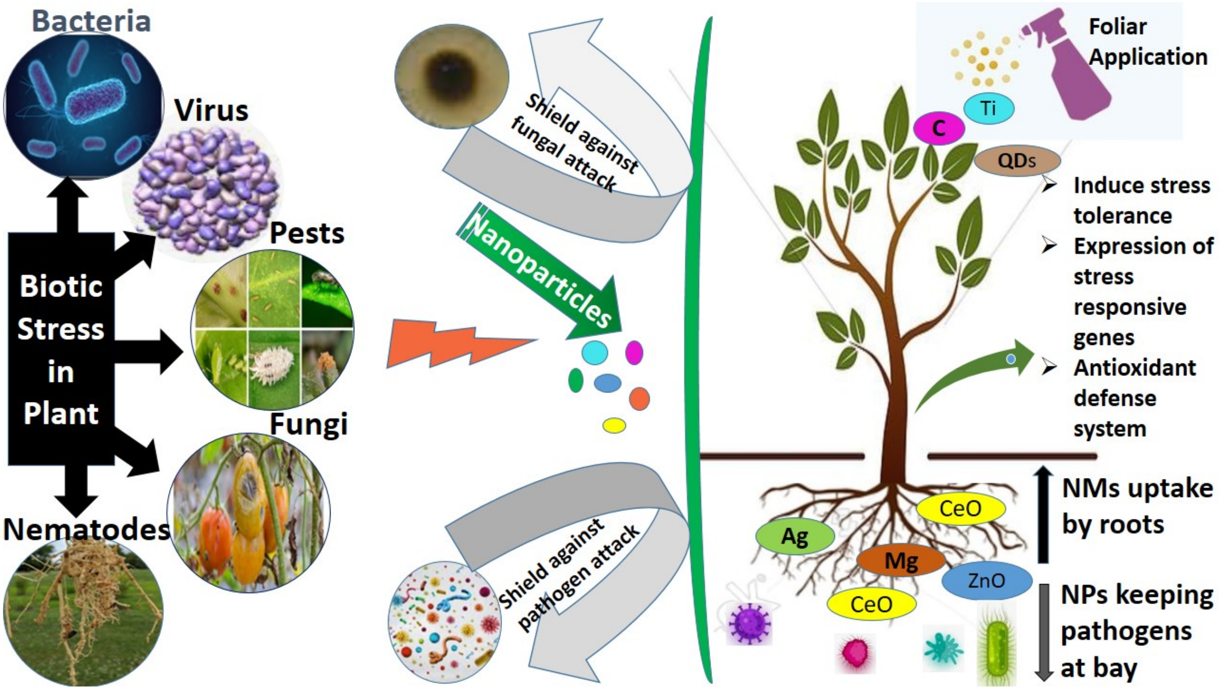


**Figure 1**. Schematic diagram of the role of nanotechnology in curtailing biotic stress in plants

## 2. Synthesis of Nanomaterials

Before discussing the role and application of nanomaterials in crop protection against biotic stresses, we first have to consider the problems associated with the formulation methods of nanomaterials. It is important to emphasize the numerous steps involved in the production of NPs, each of which can result in the contamination of the final product and adverse effects on the plants' system from these contaminants (Petersen et al., 2014). Different approaches to nanoparticle synthesis have been developed due to the indefinite applications that NPs possess in various fields of science and technology. In general, the synthesis of NPs is usuallycarried out by costly methods that harm the environment and human health (Ahmed et al., 2017 ; Mitiku and Yilma. 2018). As a result, numerous recent techniques aim to be efficient and to use eco-friendly biosynthetic approaches, e.g., using algae, fungi, other microorganisms, and plant extracts (Bora et al., 2022; Hasnain et al., 2023; Munir et al., 2023). Typically there are two approaches for synthesizing nanoparticles: the "top-bottom" and "bottom-up" approaches. Small molecules and atoms are transformed into super-small nanostructures in the bottom-up approach. In contrast, the large bulk materials are transformed into the smallest structures in the top-bottom approach. Because NPs' synthesis is complex, it necessitates specialized knowledge and equipment. After synthesis, NPs must be characterized to ensure that the desired particle's relative uniformity and size have been achieved. Plants and microorganisms hold the top positions in NPs formulation protecting the environment from toxicity (Munirt et al., 2021; Abideen et al., 2022).

**Table 1.** Synthesis of the nanoparticles by using micro-organisms (bacteria and fungi) for antimicrobial activity (Hajong et al., 2019)

| NPs | Microbes | Methods | Synthesis location | References |
|---|---|---|---|---|
| | **Bacteria** | | | |
| **Au** | *Pseudomonas aeruginosa* | Reduction | Extracellular | (Narayanan et al., 2010) |
| **Ag** | *Enterobacter cloacae* | Reduction | Extracellular | (Kalimuthu et al., 2008) |
| **Ag** | *Bacillus thuringiensis* | Reduction | Crystal spore | (Jain and Kothari. 2014) |
| **Ag** | *B. subtilis* | Reduction | Extracellular | (Saifuddin et al., 2009) |
| **Pt & Pd** | *E.coli* | Reduction | Extracellular | (Deplanche et al., 2010) |
| | **Fungi** | | | |
| **Au** | *Verticillium sp.* | Reduction | Intracellular | (Ramanathan et al., 2013) |
| **Ag** | *Aspergillus fumigatus* | Reduction | Extracellular | (Bhainsa et al., 2006) |
| **Ag** | *Fusarium oxysporum* | Reduction | Extracellular | (Durán et al., 2005) |
| **Pt** | *Neurospora crassa* | Reduction | Extracellular | (Sanghi and Verma. 2009) |
| **CdS** | *Schizosaccharomyces pombe* | | Intracellular | (Kowshik et al., 2002) |
| **CdS** | *F. oxysporum* | Enzyme mediation | Extracellular | (Rai et al., 2009) |

**Table 2.** Nanoparticles synthesis by using plant resources for antimicrobial activity (Hajong et al., 2019)

| NPs | Plants | Applications | References |
|---|---|---|---|
| **Ag** | *Olea europaea* | Antibacterial activity against drug resistant bacteria | (Khalil et al., 2014) |
| **Ag** | *Croton sparsiflorus* | Antibacterial activity against *E.coli, Staphylococcus aureus, B. subtilis* | (Kathiravan et al., 2015) |
| **$AgNO_3$** | *Argemone mexicana* | *P. aeruginosa, E. coli, A. flavus* | (Singh et al., 2010) |
| **$AgNO_3$** | *Solanum torvum* | *A. niger, A. flavus, S. aureus, P. aeruginosa* | (Govindaraju et al., 2010) |
| **ZnO** | *Camellia sinensis* | Effective against microbes | (Senthilkumar and Sivakumar. 2014) |
| **ZnO** | *Moringa oleifera* | *E. coli, P. aeruginosa, S. aureus, B. subtilis & C. tropicalis, C. albicans* | (Elumalai et al., 2015) |
| **Hexagonal & spherical ZnO** | *Parthenium hysterophorus L.* | *A. niger, A. flavus* | (Rajiv et al., 2013) |
| **CuO** | *Phyllanthus amarus* | *Effective against B.subtilis* | (Acharyulu et al., 2014) |
| **$TiO_2$** | *Psidium guajava* | *Proteus mirabilis, S. aureus, Aeromonas hydrophila, P. aeruginosa, E. coli* | (Santhoshkumar et al., 2014) |

## 2.2. Green Synthesis of Nanomaterials

Researchers are working on eco-friendly and sustainable agricultural practices for food production without affecting the environment or wasting resources (Islam et al., 2017). New technologies such as nanotechnology are essential for sustainable and environment-friendly food production (Zulfiqar et al., 2019). Green nanoparticle synthesis is becoming a very interesting topic nowadays. Due to its environmental friendliness and cost-effectiveness, using living organisms for the green synthesis of nanoparticles is a good substitute for physical and chemical processes (Parveen et al., 2016). For example, it has been proposed that using plants for synthesizing nanoparticles is more helpful than chemicals (Siddiqui et al., 2015). Biomolecules like phenolics, flavonoids, alkaloids, terpenoids, and proteins can all be involved in the green synthesis of nanoparticles; they are used to reduce metal salts(Ovais et al., 2018). Moreover, hydroxyl groups in plant amino acids, proteins, carbohydrates, and nucleic acids, contribute to the stabilization of NPs (Dorjnamjin et al., 2008).

The use of living organisms is getting more and more attention. The currently adopted chemical or physical methods are more harmful to the environment than the biological formulation of metal NPs and metal oxide NPs (Samuel et al., 2022). As a result, researchers have turned their interest to plants, expanding the scope of nanomaterials that plants synthesize on a large scale because plant-synthesized NPs are more stable than those produced by other biological systems, and also come in various shapes and sizes (Ramesh et al., 2014). Metal- or carbon-based nanoparticles can also be synthesized, as illustrated in Figure 2. Zinc oxide (ZnO), silver oxide ($Ag_2O$), titanium oxide ($TiO_2$), gold oxide ($Au_2O$), copper oxide (CuO), and cerium oxide ($CeO_2$) are metal-based engineered nanoparticles produced and utilized extensively. Mn, $Fe_3O_4$, and CaO are other widely produced and used nanoparticles (Rico et al., 2015).

Numerous plants have been used for nanoparticle synthesis. For example, the formation of ZnO NPs from *Trifolium pratense* flower extract can replace the chemical biosynthesis of these NPs. ZnO NPs exhibit a wide spectrum of activity, for example demonstrating antibacterial activity against the bacteria *Pseudomonas aeruginosa* (Dobrucka and Długaszewska. 2016). Numerous plant species, including *Aspalathus linearis* and *Moringa oleifera*, have been used for manufacturing ZnO NPs (Diallo et al., 2015 ; Matinise et al., 2017). In addition, *Sageretia thea*-based Ag NPs were highly bactericidal against gram-positive (e.g, *Staphylococcus aureus*) and gram negative-bacteria (e.g., *Escherichia coli*). Therefore, Ag NPs have great potential in the medical field, thus making *Sageretia thea* a valuable resource for nanoparticle synthesis (Sharma et al., 2013). Bio-synthesized NPs have an extraordinary potential to reduce plant stress, promote growth, and increase agricultural yield (Kaningini et al., 2022). Therefore, plant extracts are considered a cheap, environmentally friendly, and effective substitute for the large-scale production of NPs (Khatoon et al., 2017).

It is relatively uncommon to synthesize NPs with viruses. However, researchers have used the recombinant and wild-type Tobacco mosaic virus (TMV) in synthesizing silver (Ag) and gold (Au) nanoparticles(Dujardin et al., 2003;Lomonossoff and Evans. 2011). Additionally, the formulation of FeO and Fe-Pt NPs from Cowpea mosaic virus (CMV) and Brome mosaic virus (BMV) was possible (Dudhagara et al., 2022). In contrast to the physical and chemical methods currently used for nano-synthesis, nanotechnology has potential to become a green technology. It is a crucial technique emphasizing clean and environment-friendly methods (Tanwar and Sushil. 2019).

Several studies have reported the pesticidal activities of metal oxide NPs against several plant pathogens. For example, biogenically synthesized ZnO NPs from the extract of lemon peel possessed fungicidal activity against the fungus *Alternaria citri*, an organism which causes a common disease known as citrus black rot (Sardar et al., 2022). Similarly, leaf extract of *Cinnamomum camphora* containing ZnO NPs demonstrated fungicidal activity against the fungus *Alternaria alternata*, which causes early blight disease in *Solanum lycopersicum* (Zhu et al., 2021). Additionally, fungicidal activity against *Fusarium solani, Sclerotium sclerotia, Aspergillus terreus,* and *Fusarium oxysporum* was observed with ZnO NPs biosynthesized with *Penicillium chrysogenum* (Mohamed et al., 2021). Some researchers have used the photoactivation of ZnO nanoparticles as a novel strategy for plant defense. This strategy has allowed the killing of *F. oxysporum* and *Escherichia coli* in infected seeds (Zudyte et al., 2021). Furthermore, photoactivated ZnO NPs increased fruit shelf life, promoted crop production, and reduced *B. cinerea* incidences in strawberries (Luksiene et al., 2020).

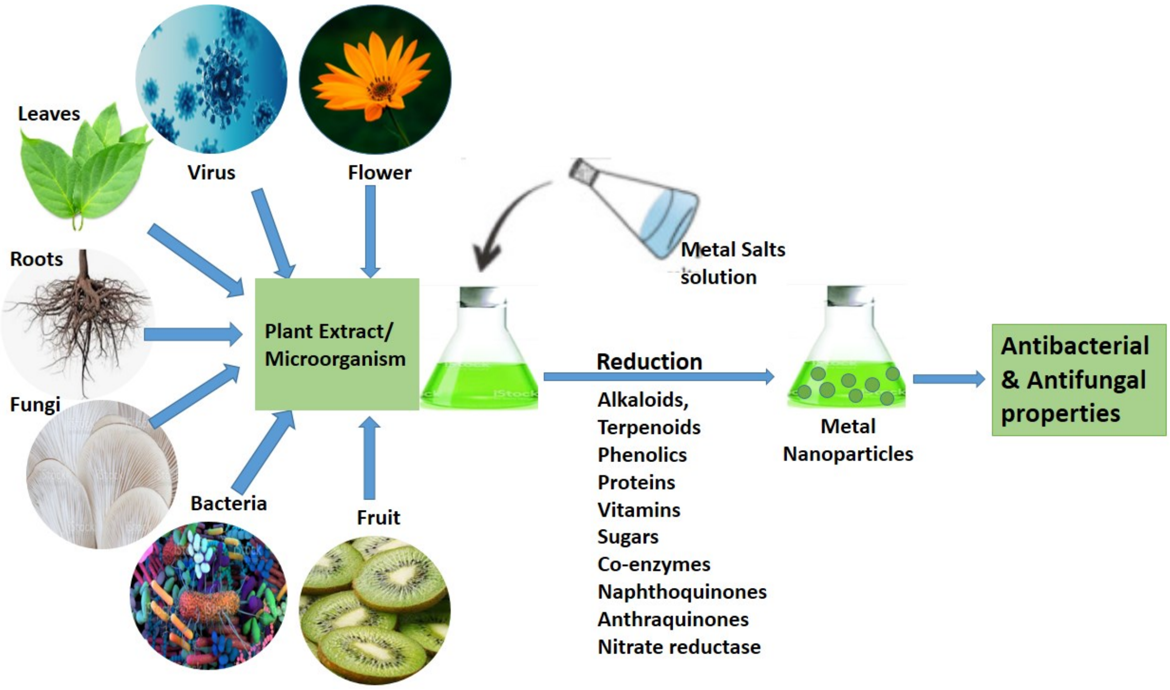


**Figure 2**. Mechanism of green synthesis of metallic nanoparticles

## 3. Nanotechnology in Disease Diagnosis and Detection

For effective disease management and the prevention of epidemics, timely diagnosis of biotic stresses is essential. Nanotechnology contributes to the development of techniques for detection that are quick, accurate, and do not require any complicated methods to operate. The application of nanotechnology to the early diagnosis, detection, and management of plant pathogens that cause diseases is known as nanophytopathology (Hussain. 2017). Different plant diseases can be diagnosed with nanotechnological tools such as nanofabrication, nanobiosensors, nanobarcodes, and other systems for diagnosis. Additionally, nanodiagnostic kits can quickly and easily identify plant pathogens, preventing the spread of epidemics. Microbe-plant interactions, gene transfer between pathogen and host, genetics of pathogen population, and hosts can all be studied using nano-molecular techniques. For diagnosing plant viruses, bacteria, and fungi, various Quantum Dots (QDs) and nanoparticles are used with a high degree of accuracy (Boonham et al., 2008 ; Qasim et al., 2022 ; Singh et al., 2022). The utilization of significant devices and nanomaterials in identifying and analyzing diseases in plants is described below.

### 3.1. Nanoscale Biosensors

Surface Plasmon Resonance (SPR), a NP-based sensor, can be used to identify baculovirus, *Autographa californica*. Nanoparticles possess physical properties which can be changed chemically, alter surface area to volume ratios, increase binding affinity with target proteins, and cause delayed or enhanced activity (Baac et al., 2006 ; Sharon et al., 2010). Yao et al. (2009) distinguished *Xanthomonas axonopodis* in solanaceous crops responsible for bacterial spot disease by fluorescent $SiO_2$ and antibodies. Nanosensors, therefore, can enhance the detection of pathogens, and plant disease diagnosis, with the aim to forecast the likelihood or intensity of the disease outbreaks. The use of a bionanosensor increased the sensitivity to identify pathogens, and the response time to identify potential disease issues decreased significantly. Utilizing nanoscale biosensors, biotic stresses like viruses, fungi, bacteria, and biohazardous and toxic materials in the environment, can be detected and quantified in minute amounts (Hajong et al., 2019). Carbon nanotubes (CNTs), quantum dots (QDs), dendrimers, superparamagnetic NPs, and some metal nanoparticles are amongst the nanosensors described by Otles and Yalcin (2010), for example. Nanosensors can detect the mycotoxins of fungi such as *Penicillium, Aspergillus, Rhizopus, Fusarium, and Alternaria*, which are produced as harmful secondary metabolites. Through improved fertiliser management, lower input costs, and environmental safety, nanoscale biosensors support high-yield andprecision farming (Duhan et al., 2017).

### 3.2. Quantum Dots (QDs)

Nanocrystals that have properties of semiconductors are known as quantum dots (QDs). Essentially, when activated by light, these nanoparticles show fluorescence. QDs are utilized as inorganic fluorophores that have the potential to detect the concentration of nucleic acids because of their unusual physical properties, such as long fluorescence periods and small emission peaks. For an extensive absorption spectrum, QDs can potentially be excited and so show all the colors using a single light source (Warad et al., 2004). The use of QDs in plant pathology has been discouraged, whereas, in medical sciences, their applications to detect certain biological markers are very promising. When isolates of *F. oxysporum* were treated by mixing in tellurium dichloride ($TeCl_2$) and cadmium chloride ($CdCl_2$) under an ambient atmosphere, then cadmium telluride quantum dots (CdTeQDs) having high fluorescence were produced (Jain. 2003). A QDs-Fluorescence Resonance Energy Transfer (FRET)-based sensor was developed to identify *Phytoplasma aurantifolia*-induced lime witches' broom disease. The QDs-FRET-based sensor was also used to recognize *Polymyxa betae,* a vector to disseminate the infection of Beet Necrotic Yellow Vein (BNYVV) (Safarpour et al., 2012).

## 3.3. Nanobarcodes

NP-based barcode or bio-barcoded DNA (b-DNA) is an extremely sensitive method for detecting and amplifying nucleic acids by pathogen tagging. Bio-barcode detection is a one-of-a-kind method that could serve as an alternative to the PCR procedure. However, only a few pathogens can be detected by these devices. By forming a multiplexed diagnostic kit, nanobarcodes can be used to assay pathogen DNA (Li et al., 2005). By oligonucleotide-modified magnetic AuNPs, bio-barcoded DNA tests can easily separate the target protein and amplify the signal (Goluch et al., 2006). Eastman et al. (2006) created a QD nanobarcode-based microbead random array platform for reproducible and accurate gene expression profiling and plant pathogen detection.

## 3.4. Nano Diagnostic Kit

The use of nano diagnostic kits are robust and simple diagnotic methods which can easily be used to detect and identify pathogens in the field. However, few studies have used the nano diagnostic kits in diagnosing plant pathogens. For example, Lattanzio et al. (2012) utilized a type of nanodiagnostic kit, multiplex dipstick immunoassay, to ascertain *Fusarium* mycotoxins, deoxynivalenol, fumonisins in wheat, oats, and maize, HT-2 and T-2 toxins and zearalenone. Further studies are needed in employing nano diagnostic kits to rapidly identify plant diseases in the field.

## 3.5. Nanofabrication

For the timely diagnosis of plant diseases in the field, nanofabrication, a nanodiagnostic tool for imaging, has the potential to visualize plant cells and tissues (Rosen et al., 2011). The imaging time, contrast, signal strength, and tissue specificity were all enhanced by this tool's modulation of the physico-chemical properties of nanoparticles. Meng et al. (2005) reported the artificial synthesis of stomata and xylem vessels using nanofabrication, in which *Xylella fastidiosa*'s pathogenicity was observed in a microfabricated xylem chamber.

## 3.6. Nanopore Sequencing System

The technology known as nanopore sequencing, also referred to as next-generation or fourth-generation DNA sequencing technology, can be used to analyze the whole genome in a matter of minutes as opposed to hours. Pathogen identification time and expense are reduced as a result. The technique uses an outside voltage displacing from one side of a nanopore to the other electrophoretically (Hajong et al., 2019). Bronzato Badial et al. (2018) found that unidentified bacterial and viral pathogens in insect and plant tissues can be detected using a portable nanopore-based system, a highly similar sequencing technique. The nanopore sequencing method can also detect small genomes of the specific insect vector microbiome strains that constitute high-titer pathogens. The most significant limitation of such cutting-edge sequencing is that costly equipment with quite difficult sample preparation and data processing is needed (Malapi-Wight et al., 2016).

### 3.7. Nanoparticles in MicroRNA Detection

Innovative technologies based on MicroRNA (miRNA) have the potential to identify and control plant diseases., miRNA, an endogenous noncoding RNA molecule of around 18–23 nucleotides, has crucial roles in the regulative processes that control the expression of genes in both animals and plants. It is a potent instrument for controlling various plant diseases (Chaudhary et al., 2018). Accurate and timely diagnosis of plant diseases can be achieved through sensitive and precise evaluation of miRNAs as biomarkers (Degliangeli et al., 2014). Microarrays and real-time reverse transcription polymerase chain reactions (qRT-PCR) are typically used for miRNA detection. Because these detection methods are prone to error, they could be replaced with more precise and sensitive methods using nanotechnology for miRNA sensing. Artificial miRNA (amiRNA) technology can be used for silencing plant genes by targeting endogenous miRNA precursors(Parizotto et al., 2004 ; Schwab et al., 2006). Targeted silencing of the desired gene is achieved through substitutions of the oligonucleotide, which possess the same intact secondary structure of precursors of endogenous miRNA(Ai et al., 2011). For example, prevention from diseases caused by plant viruses like Turnip mosaic virus (TMV) and Turnip yellow mosaic virus (TYMV) was demonstrated by the use of miRNA expression in transgenic Arabidopsis virus (TMV) (Niu et al., 2006).

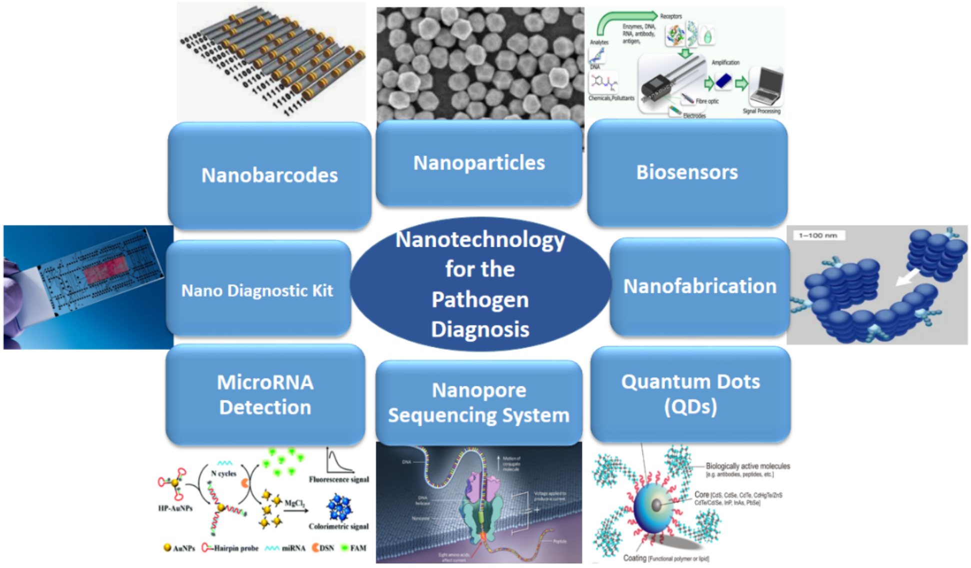


**Figure 3**. Nanotechnology in the diagnosis of plant pathogens

## 4. Nanotechnology in Plant Disease Management

Several nanotechnology applications have been developed to aid plant health and combat disease management challenges. Nanoparticles can be used in general nonbiological and biological applications, as well as multiplexed bioassays for the protection of plants. This includes the use of nanosensors, nanobarcodes and nanotubes in disease diagnosis, whereas the disease management requires nanopesticides, nanobactericides, nanofungicides, nanoinsecticides and their controlled release in agriculture (Hazarika et al., 2022). Due to their antimicrobial activity, nanoparticles and biomolecules can be utilized in numerous fields for killing disease-causing microorganisms, such as yeast, fungi, and bacteria (AbdelGawwad et al., 2020).

### 4.1. Nano-pesticides

Several pesticides on the market can be used to eliminate, control, or stop pests (Xie et al., 2019). Pesticides are categorized based on their chemical properties, mechanism of action, type of phytopathogen they attack, and their applications or uses. For example, insecticides, herbicides, and fungicides are used because of their effectiveness and wide mode of action against many plant pests and diseases (Thiour-Mauprivez et al., 2019). When applied directly, pesticides accumulate in food either in trace quantities or at high levels and therefore can enter the food chains, further facilitating biomagnification and accumulation (Tahir et al., 2019). A pesticide poses a danger not only to the person applying it in the field but also to those who consume foods contaminated with pesticide poison, which canremain functional over an extended period. However, there is currently no ideal pesticide, and the ideal applications of many insecticides, fungicides, and other pesticides are unknown (Thiour-Mauprivez et al., 2019). Evolving formulations of insecticides, pesticides, and insect repellents can be made with NMs (Gajbhiye et al., 2009). Additionally, it is suggested that validamycin-loaded Porous Hollow Silica NPs (PHSNPs) is an effective hydrophilic delivery system for water-soluble pesticides for a controlled release (Souza et al., 2019 ; Bindra and Singh. 2021).

It has been reported that chitosan NPs positively impact theplants' innate immune responses. Chitosan NPs' treatment greatly improved the plants' natural immune system by stimulating the defense enzyme reactions, upregulating the defense genes and antioxidant enzymes, as well as increasing accumulation of nitric oxide (NO) and phenolics. For sustainable organic cultivation, chitosan NPs can also be used more effectively as a disease control and phytosanitary agent than natural chitosan (Chandra et al., 2015). Sulfur nanoparticles (SNPs) were utilized by Rao and Paria (2013) as a green nanopesticide against the phytopathogens *Venturia inaequalis* and *Fusarium solani*. Furthermore, Rouhani et al. (2012) evaluated the capability of Ag NPs against *A. nerii* insects. Using the solvothermal technique, Ag and Ag-Zn NPs were formulated. *A. nerii* was then subjected to insecticidal solutions of different concentrations. For comparison, imidacloprid, a systemic insecticide of the neonicotinoids, was administered as a conventional insecticide. The finding demonstrated that for the *A. nerii* pest management program. In crop plants, nanoparticles can release active ingredients or drugs to treat all stresses. Polymeric NPs, FeO NPs, and Au NPs are just a few of the many nanomaterials that are easily synthesized and can be used as pesticides and fertilizers on plants, or drug delivery molecules in humans (Sharon et al., 2010).

## 4.2. Nano-fungicides

Metal nanoparticles, in the cultivation of plants, can be utilized as both fungicides and growth stimulants. Olchowik et al. (2017) reported spontaneous ectomycorrhizal colonization in seedlings of *Quercus robur* and the impact of Cu- and Ag-NPs on the powdery mildew-infected leaves. Spores incubated with Ag NPs showed an evident decrease in mycelial growth. Ag NPs have multiple modes of antimicrobial activity, a broad spectrum, and powerful inhibitory properties. Compared to synthetic fungicides, Ag NPs are less harmful to animals and humans, explaining their common uses over commercially available fungicides to control a variety of pathogens in plants (Malandrakis et al., 2019). Cu-containing fungicides produce hydroxyl radicals that are extremely reactive and have the potential to harm proteins, DNA, lipids, and other biomolecules (Husak and sciences. 2015).

Cu NPs were also used by Banik and Pérez-de-Luque (2017) against plant pathogens, e.g., bacteria, fungi, *Trichoderma harzianum*, *Rhizobium spp*., and Oomycetes. The authors also reported inhibited growth of *Phytophthora syringae, P. cinnamomi,* and *Alternaria alternata* when Cu NPs were integrated with non-nano Cu-like copper oxychloride (COC). It was also found that Cu NPs did not kill the beneficial *T. harzianum* or *Rhizobium spp.*, suggesting they are useful in the agroecosystem. Furthermore, ZnO NPs can be utilized as fungicides and bactericides in food and agriculture applications. For example, Xie et al. (2011) indicated that ZnO NPs improved plant growth and development, and activated the plant defense system by stimulating the production of reactive oxygen species (ROS), causing cell death. These NPs have shown much preferred microbicidal action over bulk Zn particles, with properties such as high surface-to-volume ratio and small size permitting good interaction with microorganisms.

Kim et al. (2012) also revealed the antifungal efficacy of Ag NPs against *Alternaria. brassicicola, A. alternata, Cylindrocarpon destructans, Botrytis cinerea, Cladosporium cucumerinum, A. solani, Didymella bryoniae, Corynespora cassiicola, Fusarium oxysporum f.sp. lycopersici, Glomerella cingulate, F. oxysporum f.sp. cucumerinum,* and some other strains of fungi. Similarly, Khan et al. (2021) showed the antibacterial and antifungal activities of Ag NPs against *Pseudomonas needle, Erwinia sp., Bacillus megaterium, Fusarium avenaceum, F. graminearum,* and *F. color*. According to Abdelmalek and Salaheldin (2016), Ag NPs have fungicidal activity against the fungi *Alternaria alternata, Penicillium digitatum,* and *A. citri*. Furthermore, Krishnaraj et al. (2012) reported that Ag NPs had antifungal activity against *Macrophomina phaseolina, Alternaria alternata, Sclerotinia sclerotiorum, Rhizoctonia solani, Botrytis cinerea,* and *Curvularia lunata*. Moreover, Jo et al. (2009) reported Ag NPs' antifungal activity against *Magnaporthe grisea* and *Bipolaris sorokiniana*. Divya et al. (2017) suggested that chitosan-NPs had fungicidal activity against the fungi *A. alternata* and

*Macrophomia phaseolina*. Similarly, Xing et al. (2016) also showed that chitosan-NPs possess fungicidal activity against the fungi *A. niger* and *F. solani*.

Several investigators have synthesized Au NPs and reported their antifungal activity against several plant pathogens (Table 3). For example, Jayaseelan et al. (2013) synthesized *Abelmoschus esculentus*-derived Au-NPs and reported their antifungal activity against *Aspergillus flavus, A. niger, Candida albicans,* and *Puccinia graminis* f. sp. *tritici*. In addition, the standard well diffusion method showed antifungal activity of Au-NPs against *A. niger*, *Candida albicans, A. flavus*, *Puccinia graministritci*, *P. graminis,* and *C. albicans*; the Au-NPs had the greatest inhibition zone compared to other NPs. Similarly, antifungal activities of CuO-NPs were reported against *Colletotrichum graminicola, Botrytis cinerea, Rhizoctonia solani, Colletotrichum musae, Penicillium digitatum, Sclerotium rolfsii,* and *Magnaporthe oryzae* (Huang et al., 2015). Giannousi et al. (2013) demonstrated the fungicidal action of CuO- and $Cu_2O$-NPs against the fungi *Phytophthora infestans*.

It has been reported that other metal oxide nanoparticles, such as MgO-NPs, Si-NPs, ZnO-NPs, and $TiO_2$, NPs also had antifungal activity against several types of fungi. For example, Sharma et al. (2016) found that MgO-NPs had antifungal activity against the fungus *Phomopsis vexans*. According to Derbalah et al. (2018), silica nanoparticles have antifungal properties against *Alternaria solani*. As per the findings of Akpinar et al. (2017), $SiO_2$-NPs were effective against *Fusarium oxysporum* and *Radicisi lycopersici*. In addition, Park et al. (2006) reported that *Colletotrichum gloeosporioides, Pythium ultimum, Magnaporthe grisea, Botrytis cineria, Pseudomonas syringae, Rhizoctonia solani*, and *Xanthomonas compestris* were all affected by Si-Ag-NPs' antifungal activity. Similarly, ZnO-NPs showed great fungicidal action against *Alternaria alternata, Aspergillus niger, B. cinerea, Penicillium expansum,* and *F. oxysporum* (Jamdagni et al., 2018). In addition, Shinde (2015) showed ZnO-NPs' promising antifungal activity against *Aspergillus fumigates* and *A. flavus*Further, ZnO-NPs had fungicidal activity against *A. flavus, A. fumigates, A. niger, Fusarium oxysporium,* and *F. culmorum*. Gunalan et al. (2012) discovered that ZnO-NPs have great antifungal activity against *Rhizopus stolonifer, A. flavus, Trichoderma harzianum,* and *A. nidulansv*. Dimkpa et al. (2013) have reported that ZnO-NPs have antifungal activity against the fungus *Fusarium graminearum*. Moreover, the fungicidal activity of $TiO_2$ NPs was reported against *F. oxysporum f.sp. radicis lycopersici*. Similarly, Hamza et al. (2016) discovered $TiO_2$-NPs had fungicidal activities against *Cercospora beticola*. Kasemets et al. (2009) found that ZnO- and $TiO_2$-NPs have fungicidal activity against *Saccharomyces cerevisiae*.

### 4.3. Nano-bactericides

For their antibacterial and antiviral properties, metal nanoparticles such as Ag, Cu, ZnO, and $TiO_2$ have been extensively studied (Padmavathi and Anuradha. 2022). For example, the bactericidal effect of Ag-NPs was reported against *E. coli* (Rodríguez-Serrano et al., 2020), *Klebsiella pneumonia* and *Staphylococcus aureus* (Hussein et al., 2019), *Bacillus subtilis* and *Escherichia coli* (Shehzad et al., 2018). In addition, Mohanta et al. (2017) found that food-borne pathogenic bacteria, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *E. coli,* were inhibited by Ag-NPs. Shahryari et al. (2020) reported that *Pseudomonas syringae* bacteria were inhibited by Ag-NPs and an Ag–chitosan composite. Au-NPs also showed bactericidal action against *E. coli* (Dang et al., 2019). Other metal oxide NPs showed bactericidal activities against several types of pathogenic bacteria. These include Cu composites against bacteria *Xanthomonas euvesicatoria* (Fan et al. (2021), MgO-NPs against *Ralstonia solanacearum* (Sharma et al., 2016), and *R. solanacearum* (Imada et al., 2016), ZnO-NPs against *E. coli* (Attar and Yapaoz. 2018), *P. aeruginosa, A. flavus, A. niger, C. albicans* (Jayaseelan et al., 2012).

ZnO-NPs, pure or doped with Fe, Mn, Cu, or Ni elements, halted the disease spread caused by the bacteria *Pantonea ananatis* in corn; when the follicle application of nanomaterial was carried out on plants before and after inoculation with the bacteria (Mamede et al., 2021). ZnO-NPs also proved effective in suppressing the bacterial blight diseases in pea plants caused by *P. syringae* and *M. incognita* (Kashyap and Siddiqui. 2022). In addition, amending the soil with ZnO-NPs improved rhizospheric microbial diversity, stimulated antioxidant response and plant growth in tomato plants, and decreased the occurrence of diseases caused by *Ralstonia solanacearum* (Jiang et al., 2021). ZnO-NPs made from *Matricaria chamomilla* flower extract had bactericidal action against *R. solanacearum* and reduced bacterial wilt disease in tomato plants (Khan et al., 2021). Likewise, ZnO-NPs derived from *Citrus medica* peel were found to be effective against *Bacillus subtilis, Streptomyces sannanesis, P. aeruginosa, Aspergillus niger,* and *Candida albicans* (Keerthana et al., 2021). Moreover, biogenic ZnO-NPs synthesized from *Trichoderma reesei, T. harzianum*, and co-culture (Shobha et al., 2020), and the strain Sx3 of *Paenibacillus polymyxa* (Ogunyemi et al., 2020), were used to halt the growth of *Xanthomonas oryzae pv. oryzae* bacteria, the causative agent of bacterial leaf blight in rice. Those authors reported improved plant growth and decreased bacterial leaf blight diseases in foliar-sprayed plants. Furthermore, several studies indicated that $TiO_2$-NPs suppressed sugar beet infection (causative agent: *P. syringae pv. aptata*) (Hamza et al., 2016), apple scab disease (causative agent: *Venturia inaequalis*), *Fusarium* wilt diseases in tomato and potato plants (causative agent: *F. solani*) (Boxi et al., 2016), bacterial blight on *geranium* and leaf spot on poinsettia (causative agents: *Xanthomonas hortorum pv. pelargonii, poinsettiicola, Xanthomonas axonopodis pv. Poinsettiicola*) (Cui et al., 2009 ; Norman and Chen. 2011), root and stem rot in sweet potatoes (causative agent: *Dickeya dadantii*) (Hossain et al., 2019).

### 4.4. Nano-nematicides

Parasitic plant nematodes account for nearly 20% of crop losses. Nematodes negatively impact yield production, particularly in subtropical and tropical regions (Sasser. 1987 ; Gohar and Maareg. 2005). The most detrimental are root-knot nematodes (RKNs), *Meloidogyne spp*., with more than 100 documented species (Trinh et al., 2019). RKNs cause an estimated $100 billion loss annually worldwide (Khan et al., 2008). Due to their large host range and high reproductive potential, RKNs are difficult to control (Hussain et al., 2016). Conventional methods to control nematodes include leaving land fallow, cultivating resistant plant varieties, crop rotation, chemical nematicides, etc. Nematicides, which are extremely toxic and harmful to the environment, but are used to control important plant-parasitic nematodes (Bhau et al., 2016). Due to their multi-site mode of action, NPs have been proven effective nematicides against numerous parasitic plant nematodes. For example, Au- and Ag-NPs have better nematicidal activity than harmful and synthetic nematicides (Thakur and Shirkot. 2017). The use of readily available nanotechnology materials is increasing, which offers promising results in controlling plant diseases caused by RKNs such as *M. incognita* (Sharon et al., 2010). Mortality of *M. incognita* J2 significantly increased after applying Si-NPs. However, it was reported that silicon carbide NPs (SiC-NPs) at a concentration of 172 mg/L and a size of 50 nm neither killed *M. incognita* J2 nor its eggs. Conversely, first-stage *C. elegans* larval growth was greatly affected by SiC-NPs (Al Banna et al., 2018 ; El-Ashry et al., 2022). Furthermore, ZnO-NPs have antimicrobial properties against various plant pathogens, including fungi, bacteria and the nematode *Meloidogyne incognita* (Elmer and White. 2018 ; Şahin et al., 2021 ; Thounaojam et al., 2021). Similarly, nematodes and viruses can be effectively treated with $TiO_2$-NPs (Kumar et al., 2022). In tomato plants, $TiO_2$ was found to be nematicidal against the RKN nematode *M. incognita* (Ardakani. 2013). Additionally, tomato plants infected with *Bactericera cockerelli* (Sulc), demonstrated an insecticidal effect from $TiO_2$-NPs. After 24 hours, $TiO_2$-NPs killed 93% of the insects when the NPs were used at concentrations above 100 ppm.

## 5. Mechanisms of Nanoparticles-Plant Interaction in Response to Biotic Stresses

In addition to gene regulation and provision of micronutrients to plants (Nair et al., 2014 ; Liu and Lal. 2015), NPs also interfere with various metabolic functions and oxidative processes (resulting in an oxidative burst).

### 5.1. Nanoparticles’ Direct Attachment to Plant Pathogens

The direct attachment of nanoparticles with plant pathogens is a well-recognized mechanism that explains the eradication of the pathogens by the NPs. For example, Ag-NPs that became attached to the *F. oxysporum* spores directly were able to penetrate the plasma membrane of the cell and disturb its permeability and respiratory mechanism of the cell (Panáček et al., 2006), and also proved fatal for the spores of fungi (Abkhoo and Panjehkeh. 2017). The amount of surface area available for interaction determines how well NPs bind to bacteria. Therefore, smaller particles having a larger relative surface area to interact with pathogens will have greater antibacterial activity than larger particles. Ag-NPs also caused DNA damage in fungal and bacterial cells. It was found that MgO-NPs adhered directly to the membranes of *Ralstonia solanacearum* cells, injured cell membranes, and decreased the cells’ motility and biofilm formation (Cai et al., 2018). Similarly, the hyphal walls of three sclerotium-forming fungi (*Rhizoctonia solani, Sclerotinia sclerotiorum,* and *S. minor*) treated with Ag-NPs were severely damaged, causing hyphal plasmolysis (Min et al., 2009 ; Cai et al., 2018). Wang et al. (2014) reported that carbon nanomaterials (CNMs) had a three-step antifungal mechanism: (a) NPs get deposited on the *F. graminearum* spores cell membranes, (b) water intake was inhibited, (c) and spore plasmolysis occur as a result. Along with the CNMs, clusters of graphene oxide (GO) and reduced graphene oxide (rGO) also wrapped on *F. graminearum* spores ( Wang et al., 2014).

### 5.2. ROS Production (Destructive or Signaling Role)

Plants exposed to biotic stress produce ROS that causes DNA damage, lipid peroxidation, and amino acid oxidation. Such oxidative stress upregulates genes encoding antioxidant enzymes, leading to plant stress tolerance development (Figure 4). When NPs are present in large quantities, they stimulate the production of ROS and interrupt mitochondrial electron transport (Cvjetko et al., 2017). Several authors have reported that NPs-plants interaction increased protein modifications, lipid peroxidation, and DNA damage (Van Breusegem and Dat. 2006 ; Atha et al., 2012 ; Garcia-Caparros et al., 2021 ; Sharma et al., 2021). The primary cause of the antimicrobial action is the production of ROS, mostly stimulated by ZnO- and Ag-NPs (Hwang et al., 2012 ; Xue et al., 2014). Deformity in the fungus cell wall and cell death happened when zinc nitrate-derived ZnO-NPs were used to combat *A. fumigatus* (Patra and Goswami. 2012). According to Zheng et al. (2005), spinach chlorophyll "a" increased by 45% following seed treatment with $TiO_2$-NPs. They discovered that this was brought about by a rise in inorganic nutrient absorption, which increased the use of the organic substance, and neutralized oxygen-basedfree radicals. NPs affected plant metabolism as well as the hormonal balance of plants. For example, Ag-NPs increased the cytokinin levels in *Capsicum annuum* (Vinković et al., 2017), and CuO-NPs decreased abscisic acid (ABA) and indole-3-acetic acid (IAA) in cotton (Le Van et al., 2016). It seems clear that NPs can induce ROS production in plants. ROS often have destructive properties but they also play signaling roles in managing environmental stress tolerance. The equilibrium between ROS production and accumulation, and ROS scavenging, is necessary to perform their dual function. As a result, antioxidative mechanisms developed by plant cells allow them to regulate the level of ROS. Ascorbate, glutathione, carotenoids, phenolics, and tocopherols, are examples of non-enzymatic molecules; and catalase, superoxide dismutase, guaiacol peroxidase are examples of enzymatic molecules that have antioxidant actions for scavenging ROS produced in plants exposed to NPs (Sharma et al., 2012 ; Raza et al., 2022). Several researchers have reported the production of ROS and confirmed that NP-plant interactions regulate the antioxidant systems (Faisal et al., 2013 ; Jiang et al., 2014 ; Da Costa and Sharma. 2016). Plants will eventually die of apoptosis or necrosis due to increased ROS production and accumulation if the antioxidants cannot control them. Resistance genes induce defense mechanisms, accumulating proteins, antioxidants (non-enzymatic and enzymatic), and defensive metabolites when the oxidative stress level is below the toxic threshold (Van Aken. 2015). For example, Corral-Diaz et al. (2014) reported that exposure to $CeO_2$-NP increased the accumulation of antioxidants in plants.

The important ROS produced in organisms exposed to NPs include peroxyl, hydroxyl, alkoxyl, hydroperoxyl, hydrogen peroxide, and nonradicals (Khan et al., 2021). These ROS escalate the degree of oxidative pressure, pushing the intracellular redox potential to be more positive. In addition, oxidative stress causes damage by breaking single or double-strand, disrupting the structure of pentose sugars and nitrogenous bases (De Filpo et al., 2013), causing cell membrane damage, leakage of cytoplasmic material, and alteration of nucleic acids and proteins (Ogunyemi et al., 2020 ; Zhu et al., 2021). Besides, the proton motive force (PMF) is disrupted when NP accumulate in the membranes of fungi or bacteria, causing changes in cell membrane permeability (De Filpo et al., 2013).

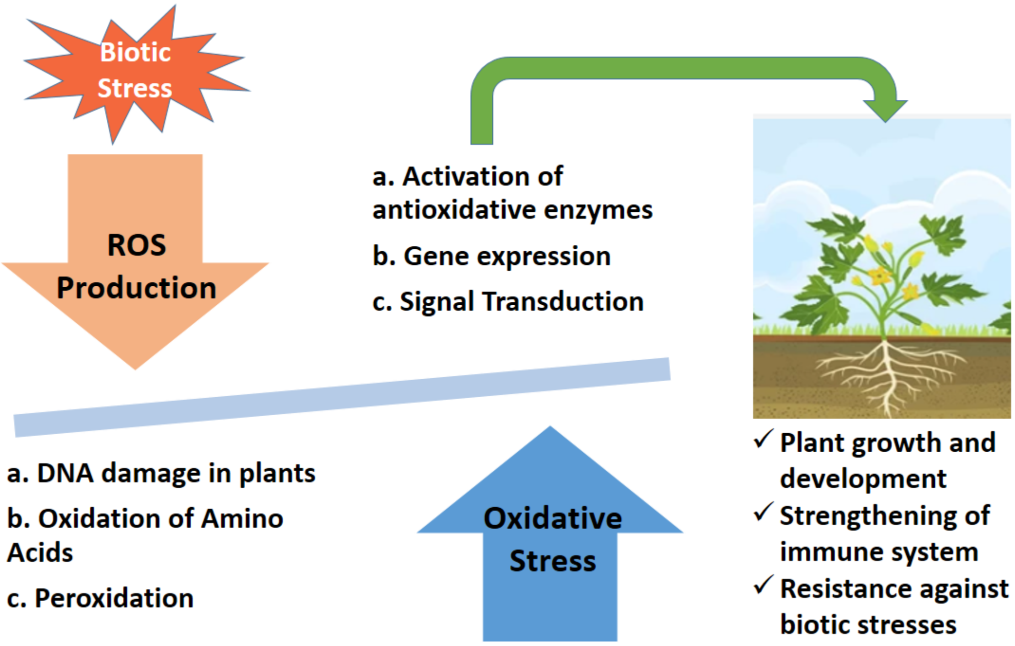


**Figure 4**. Schematic illustration of the production of ROS species in plants exposed to biotic stress and the development of stress tolerance mechanisms.

### 5.3. Other Mechanisms

Silver $Ag^+$ ions are bound to the proteins on cell membranes that contain cysteine, they cause biochemical and physiological damage (Ocsoy et al., 2013). Ag-NPs damage the plasma membrane of plant disease-causing fungi through penetration. He et al. (2011) found that ZnO-NPs caused a disturbance in the cellular machinery of *Botrytis cinerea* and *Penicillium expansum*. In addition, various NPs decreased ATPase activity at the cell level and reduced the membrane potential. The transmembrane permeability, energy metabolism, and electron transport chain are disrupted when $Ag^+$ inactivates the thiol groups in the fungus cell wall. Some other mechanisms include cell lysis, reduction in membrane permeability, enzyme complex dissociation, and fungal DNA mutations (Velmurugan et al., 2009). Ag-NPs are toxic to nematodes because they disrupt cellular mechanisms, affecting ATP synthesis and membrane permeability triggering oxidative stress, and increasing ROS production (Ahamed et al., 2010 ; Lim et al., 2012). Silver oxide ($SiO_2$) NPs cause *Caenorhabditis elegans* to prematurely age by reducing pharyngeal pumping and cause the accumulation of nuclear amyloid and insoluble ubiquitinated proteins (Scharf et al., 2013). Li et al. (2017) found that *Sclerotinia homoeocarpa* exposed to Ag-NPs and ZnO-NPs induced stress response genes expression, such as superoxide dismutase 2 (ShSOD2) and glutathione *S*-transferase (Shgst1), as well as an increase in the nucleic acid content of fungus hyphae. It was also discovered that

Ag-NPs accumulation into *S. homoeocarpa* cells is aided by a Zn transporter known as Shzrt1. MWCNT disrupted the expression of many genes for water channels in tomato plants, including LeAqp2 (Khodakovskaya et al., 2011). Malerba and Cerana (2016) gave the summary of potential mechanisms for the microbicidal effects of chitosan, including cell membrane disruption, agglutination, halting microbial growth and reducing H$^+$-ATPase activity, formation of toxins and protein synthesis, and causing a blockade of mineral nutrient flow channels.

The sequence-specific gene silencing via RNA interference (RNAi) pathway discovery has ushered in novel strategies for controlling pathogens and pests (Figure 5). It is a natural process for gene regulation and defense against various pests. RNAi mechanisms play a crucial role in the growth of plants and resistance against viruses and host development. It can also be used to target weeds, viruses, fungi, and pests (Borges and Martienssen. 2015). The general mechanism by which double stranded RNA (dsRNA) is applied to the RNA of a pathogen is shown in Figure 5. Dicer-like (DCL) enzymes processed dsRNA into small-interfering RNA (siRNA) in plants, triggering RNAi. The RNA Induced Silencing Complex (RISC), contains these siRNAs. The pathogen RNA is prevented from being used as a translation template by the presence of siRNAs, directing the RISCs to base pairs for degradation (Ahsan et al., 2021). Since its discovery, the RNAi mechanism has become an effective genetic modification method for combating plant pathogens and pests (Mitter et al., 2017). However, the generation and use of genetically modified organisms are contentious and subject to stringent regulations in most nations. As a result, new dsRNA delivery strategies are the subject of investigation (Worrall et al., 2018).

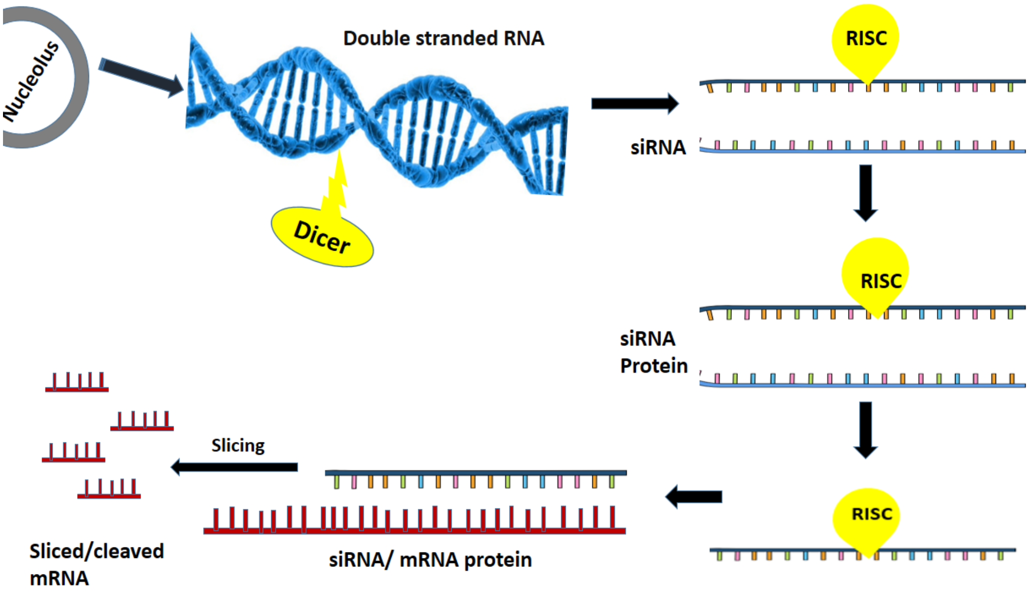


**Figure 5.** Illustration of the RNAi pathway overview

## 5.4. Mechanisms of NPs-Insect Interaction

The antimicrobial mechanism of NPs is ambiguous, but more than one potential system may occur simultaneously at the same time. The nanoparticles protect plants in two ways: first, they serve as a pesticide carrier that can be sprayed on the plants; second, the crop's protection and high yield are provided by the nanoparticles. Nanoparticles acting as carriers have the potential to provide several advantages, including an increase in the pesticides' shelf life, an increase in the solubility of poorly soluble pesticides, and an increase in the site-specific intake of a target pest (Jalil and Ansari. 2020). For protection against pathogens and pests, nanoparticles can also be directly applied to plant seeds, roots, and foliage. For their antibacterial and antifungal affinity, metal NPs, such as Cu, Ag, $TiO_2$, and ZnO, have been the subject of extensive research. *Alternaria alternata, Rhizoctonia solani,* and *Sclerotinia sclerotiorum* have all been shown to be inhibited by Ag NPs (Krishnaraj et al., 2012). When poly-dispersed Au-NPs were induced into the plant through mechanical abrasion, they disintegrated the barley yellow mosaic virus particles, protecting the host. For the detection of pathogens, DNA-Au NPs probes are promising as a new class of biosensors. It has been reported that insect pests protect their water content with cuticular lipids, preventing desiccation-caused death. Pest insects die when the plant surface is treated by nanosilica as an insecticide because the particles are absorbed by physisorption into the cuticle lipids (Barik et al., 2008). Nanotubes containing aluminum silicate ($Al_2SiO_5$) can adhere to plant surfaces and insect pests' surface hairs, disrupting physiological processes (Patil. 2009). Besides, the feeding preference of *Spodoptera littoralis*, the African cotton leafworm, is also influenced by nanosilica, which increases tomato plant resistance. Nanosilica also impacts the insect’s biological parameters, reducing its reproductive potential in longevity and nymph production (El-Bendary and El-Helaly. 2013).

## 6. Nanomaterials used in Disease Management

The nanoparticles synthesized from nonmetals, metalloids, metal oxides, and carbon have microbicidal activity. Some of these nanoparticles increase the resistance to biotic stress and have nutritional benefits to plants. The nanoparticles can also strengthen the immune system of plants (Mittal et al., 2020). The nanoparticle commonly used as carriers for fungicides, insecticides, and herbicides are summarized in Table (3).

Table 3. Nanomaterials used in plant disease management (Khan et al., 2021)

| Nanoparticles | Pathogenic Species | Effects | References |
|---|---|---|---|
| Ag NPs | *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus* | Antibacterial activity | (Hussein et al., 2019 ; Rodríguez-Serrano et al., 2020) |
| Ag NPs | *Cylindrocarpon destructans, Corynespora cassiicola, Fusarium oxysporum f.sp. cucumerinum, F. oxysporum, Fusarium sp, P. spinosum, Didymella bryoniae, Glomerella cingulata, Monosporascuscannonballus, A. brassicicola, Alternaria alternata, Cladosporium cucumerinum, A. solani, Stemphylium lycopersici, F. oxysporum f. sp. lycopersici, Macrophomina phaseolina, Magnaporthe grisea, Bipolaris sorokiniana, Botrytis cinerea,* | Antifungal activity | (Kim et al., 2012) |
| Ag NPs | Gram-positive (*Bacillus subtilis*), Gram-negative (*Escherichia coli*) | Bactericidal effect on tested bacteria | (Shehzad et al., 2018) |
| Ag NPs | *Penicillium digitatum, Alternaria. citri, A. alternata,* | Antifungal properties | (Salaheldin. 2016) |
| ZnO NPs | *Fusarium graminearum* | Antifungal activity | (Dimkpa et al., 2013) |
| ZnO NPs | *Aspergillus nidulans, A. flavus, Trichoderma harzianum, Rhizopus stolonifer,* | Antifungal activity | (Gunalan et al., 2012) |
| ZnO NPs | *A. niger, F. oxysporium, A. fumigatus Fusarium, Aspergillus flavus and culmorum* | Higher antifungal activity against *A. flavus* | (Rajiv et al., 2013) |
| ZnO NPs | *Aspergillus fumigates, Aspergillus flavus* | Antifungal activity | (Shinde. 2015) |
| ZnO NPs | *Alternaria alternate, A. niger, Botrytis cinerea* | Antifungal activity | (Jamdagni et al., 2018) |
| $TiO_2$ NPs | *Cercospora beticola* | Pathogen growth was inhibited | (Hamza et al., 2016) |
| $TiO_2$ NPs | *Meloidogyne incognita* | Controlled *M. incognita* | (Ardakani. 2013) |
| $TiO_2$ NPs | *F. oxysporum f. sp. lycopersici, F. oxysporum f. sp. radiceslycopersici* | Antifungal activity | (Sar and Unal. 2017) |
| $TiO_2$ NPs & ZnO NPs | *Saccharomyces cerevisiae* | Antifungal activity | (Kasemets et al., 2009) |
| $TiO_2$ NPs | *P. cubensis, P. syringaepv. lachrymans* | Reduced infection of the pathogen | (Cui et al., 2009) |
| Nano Si-Ag | *Rhizoctonia solani, Botrytis cineria, Pythium ultimum, Magnaporthe grisea, Pseudomonas syringae,* | Show antibacterial and antifungal activity | (Park et al., 2006) |
| $SiO_2$ NPs | *F. oxysporum f. sp. radicislycopersici, F. oxysporum f. sp. lycopersici* | Antifungal properties | (Akpinar et al., 2017) |

| | | | |
|---|---|---|---|
| SilicaNPs | *Alternaria sp* | Antifungal activity | (Derbalah et al., 2018) |
| MgO NPs | *Phomopsis vexans and Ralstonia solanacearum* | Antibacterial and antifungal activity | (Sharma et al., 2016) |
| Cu composites | *Xanthomonas euvesicatoria* | Antibacterial activity | (Fan et al., 2021) |
| CuO and $Cu_2O$ NPs | *Phytophthora infestans* | Antifungal activity | (Giannousi et al., 2013) |
| CuO NPs | *Botrytis cinerea, Rhizoctonia solani, Magnaportheoryz* | Antifungal activity | (Huang et al., 2015) |
| Au NPs | *Aspergillus flavus, A. niger, Candida albicans, Puccinia graminis tritci* | Antifungal activity | (Jayaseelan et al., 2013) |
| Au NPs | *Puccinia graminis tritci, Escherichia coli* | Antibacterial activity | (Dang et al., 2019) |
| Au and ZnO NPs | *Escherichia coli* | Antibacterial activity | (Attar and Yapaoz. 2018) |
| AuNPs | *Staphylococcus aureus, E. coli* | Antibacterial activity | (Yuan et al., 2017) |
| AuNPs | *Candida albicans* | Antifungal activity | (Aljabali et al., 2018) |
| $TiO_2$ NPs | *X. axonopodis pv. Poinsettiicola, Xanthomonas hortorum pv. pelargonii* | Antibacterial activity | (Norman and Chen. 2011) |
| ZnO NPs | *Botrytis cinerea, Xanthomonas hortorum pv. pelargonii* | Significantly inhibit growth | (He et al., 2011) |
| ZnO NPs | *Ralstonia solanacearum* | Antibacterial activity | (Khan et al., 2021) |
| ZnO NPs | *Fusarium oxysporum, Aspergillus niger* | Antibacterial and antifungal activity | (Patra et al., 2012) |
| ZnO NPs | *Rhizopus stolonifer, Aspergillus niger and Mucor plumbeus* | Inhibit germination of spores of fungi | (Wani and Shah. 2012) |
| ZnO NPs | *Escherichia , Botrytiss,* | Antibacterial and antifungal activity | (Kairyte et al., 2013) |
| Metallic NPs | Bacteria and Fungi | Antifungal and antibacterial activity | (Slavin et al., 2017) |
| Metallic NPs | Microbes | Antifungal and antibacterial activity | (Singh et al., 2019) |
| Chitosan NPs | *Streptococcus* | Antibacterial activity | (Chávez de Paz et al., 2011) |
| ZnO NPs | *Penicillium expansum , Fusarium oxysporum* | Antifungal activity against the tested fungal species | (Jamdagni et al., 2018) |
| CuO NPs | *Colletotrichum graminicola, Colletotrichum musae,* | Antifungal activity | (Huang et al., 2015) |
| Nano Si-Ag | *Xanthomonas compestris pv. vesicatoria, Colletotrichum gloeosporioides,* | Antifungal and antibacterial activity | (Park et al., 2006) |

## 7. Stability of Nanomaterials Used in Plant Protection

Due to their size and surface characteristics, conventional and nanotechnology-based formulations differ significantly. Because they are more effective, pesticide nanodelivery techniques such as nanoencapsulated, nanocontainers, and nanocages reduce pesticides released into the atmosphere and accelerate pesticide decomposition in soils (Sundarraj and Ranganathan. 2019). NMs' higher stability and better ability to dissolve in water are two properties that increase the pesticides' potential. Nanoformulations are regarded as excellent agrochemicals with a long shelf life that improves pesticide bioefficacy. Various nanoformulations, including nanogels, nanoemulsions, nanoencapsulations, and nanosuspensions, can be used for agrochemicals (Okey-Onyesolu et al., 2021).

### 7.1. Nanogels

Nanogels are defined as monomeric or copolymerized nanosized hydrogel systems. In addition, they can be polymeric (Phillips et al., 2010). With the emergence of nanotechnology, there is a need to create nanogel systems that have greater efficiency in delivering active components in a sustained, controlled, and targetable way. Initially, the gels appeared as semisolid formulations containing fluids and drugs in three-dimensional organic systems. Due to specific delivery system anticipation, nanosized hydrogel and microgel have become important. Nanogels are classical formulations whose volume fraction and solvent quality can vary to produce a three-dimensional structure (Kageyama et al., 2008).

### 7.2. Nanoemulsions

The nanoemulsions have good stability, dispersity, viscosity, and transparency, making them advantageous in various pharmaceutical, food, cosmetics, and agrochemical industries (Nair et al., 2010). They are transparent and kinetically stable because their particle size is <200 nm. Due to the low concentration of surfactants, pesticide formulation with nanoemulsions is more environmentally friendly and economically viable than surfactants and microemulsions (Hazra et al., 2013). Low-energy emulsification techniques are used to create nanoemulsions, and the stored energy may enable NPs of smaller sizes to last longer (Bur). Nanoemulsions, oil or water-based, contain uniformity in the suspensions of nanoparticles that kill pests and therefore have a lot of potential uses for controlling a range of diseases and pests. Nanoemulsions exhibit greater stability and increased leaf covering due to low surface tension (Gogoi et al., 2009). Although they are primarily developed for poorly water-soluble pesticides, the main benefits include hydrophobic pesticides' solubilization, absence of precipitation, enhanced uptake and increased stability.

### 7.3. Nanoencapsulation

Pesticide delivery is often dependent upon certain conditions, such as temperature and moisture. Nanoencapsulation methods provide altered pesticides, controlling and managing pesticide release and crop availability. Nanoencapsulated pesticides have the potential to withstand harsh environmental processes (evaporation, photolysis, leaching, hydrolysis, and microbial degradation) that help conventionally applied pesticides to degrade, and therefore NPs allow a small amount of pesticide to be applied effectively over a specific period of time (Eerikäinen et al., 2003).

### 7.4. Nanosuspensions

Nanosuspension is a submicron colloidal pesticide dispersion made of pure particles that surfactants stabilize. Utilizing nanosuspension formulation can address distribution issues associated with pesticides that have low solubility in water and lipids. Compounds soluble in oil but insoluble in water are treated with nanosuspensions. Pesticides are usually manufactured using liposome emulsion systems, which are water-insoluble but oil-soluble. Nanosuspensions are preferred because the lipidic nanosuspension formulation strategies used to make conventional pesticides are not suitable. Similarly, lipidic systems are not utilized when pesticides are water-insoluble and organic media are insoluble. Instead, these nanosuspensions are a good formulation strategy for pesticides removal from the soil (Nuruzzaman et al., 2019).

### Gene expression induced by nanoparticles in plants:

Transcriptional studies of the effects of NMs on plants revealed changes in gene expression in response to both biotic and abiotic stimuli(Abideen et al., 2022a). Kaveh et al. (2013) conducted a genome microarray study in which they showed that when *A. thaliana* was exposed to Ag-NPs plant growth increased at low doses, i.e., less than 2.5 mg $L^{-1}$, and decreased at higher doses, i.e., less than 5 mg $L^{-1}$. Genes responsible for hormonal stimuli as well as for pathogen detection were down-regulated at a moderate level of 5 mg $L^{-1}$. Furthermore, genes differentially expressed in response to Ag NPs and soluble $Ag^+$ shared a significant amount of gene expression, indicating that Ag NPs-induced stress was partly caused by Ag toxicity and specific nano-sized effects. Photosynthetic pathways were also impacted by NMs, according to some other reports. Ma et al. (2013) investigated the effects that $CeO_2$ and $In_2O_3$ had on *A. thaliana*. The results revealed that $CeO_2$-NPs harmed plant growth and chlorophyll production at concentrations of less than 1000 mg $L^{-1}$, but not by $In_2O_3$-NPs. Ze et al. (2011) tried to comprehend the beneficial effect of $TiO_2$-NPs on plant growth and analyzed susceptible plants' photosynthetic efficiency. The chloroplast light absorption efficiency increased, and the thylakoid membrane LHCII content increased due to plant exposure to $TiO_2$-NPs. Marmiroli et al. (2014) examined the transcriptomic response of wild-type and tolerant dissociation (Ds) transposition-induced mutant lines of *A. thaliana* when subjected to CdS-QDs. In wild-type plants, CdS-QDs prevented germination and growth, but not by releasing $Cd^{2+}$ ions. Overexpression of genes involved in defense response, SAR, and pathogenesis was seen in all lines when exposed to QDs at levels below the lethal limit. Upregulation of genes involved in synthesizing storage and lipid transport proteins (playing a role in stress response) was linked to the tolerance of one mutant line (atnp01). A member of the MYB transcription factor superfamily known to be involved in development and metabolism regulation, as well as in response to biotic and abiotic stress, was suggested to be associated with the tolerance of the second mutant line (atnp02). The accumulation of oxidized glutathione, a sign of oxidative stress, and the reduction in plant biomass of *Triticum aestivum* followed exposure to Ag-NPs (Dimkpa et al., 2013). Moreover, overexpression of a metallothionein suggested that plants responded to Ag NPs exposure by metal particle sequestration. In two studies with *Nicotiana tabacum*, exposure to $Al_2O_3$- and $TiO_2$-NPs led to a variety of phytotoxic effects (such as a decrease in plant biomass and germination rate) and an increase in a collection of miRNAs (Frazier et al., 2014).

The beneficial and detrimental effects of CNMs on various plant species have been the subject of several published studies. Shen et al. (2010) reported that SWNTs caused cell death, DNA damage, and the production of ROS in *Oryza sativa* protoplast cells. Expression of ascorbate peroxidase and SOD, two ROS-scavenging proteins, was up-regulated in exposed leaf cell cultures of *A. thaliana*, confirming the involvement of ROS in NP responses. Khodakovskaya et al. (2012) reported that MWNTs significantly increased the growth of *N. tabacum* cell cultures across a wide concentration range and induced several genes involved in cell division (cell cycle-CycB), cell wall formation (extension, NtLRX1) and water transport (aquaporin, NtPIP1). In the same way, Lahiani et al. (2013) reported that *Hordeum vulgare*, *Glycine max*, and *Zea mays* were more likely to germinate and grow when exposed to MWNTs. This was linked to the overexpression of genes encoding various types of water channel proteins. Many studies suggest that SWNTs, like other types of stress, initiate an epigenetic response (Yan et al., 2013). Plant exposure to NMs appears to elicit a broad molecular response, affecting multiple transcription factors and genes involved in various cellular stresses such as biotic and abiotic stimuli.

## 8. Eutrophication and Toxicity of Nanomaterials (NMs) Used for Disease Management

Nanotechnology has numerous life-saving applications for humankind but on the other hand may adversely affect the ecosystem and the environment depending upon the concenrtarion. The shape, size, and dose of nanoparticles, their types, concentration, and exposure duration all play an important role in how nanoparticles affect organisms, whether they are microbes, plants, or animals. The following are some of the restrictions and potential dangers that come with the nanomaterials released into the environment:

1. The accumulation of nanoparticles in food, water, and agriculture harms humans, the environment, plants, and animals (Gruère et al., 2011).
2. Microbial populations are sensitive indicators of the soil's quality and changes caused by contamination and external stress (Sharma et al., 2010). Metal-based nanoparticles generally appear more toxic to the soil microbial community than organic NPs. Even at

very low concentrations, metal-based NPs are said to alter enzymatic activities (Simonin et al., 2015).

3. Intentional excessive application of NMs in agriculture to treat diseases is one way that NMs come into contact with plants. As a result, NMs may accumulate and spread among species via food chains due to their persistent introduction into plants, posing a threat to the ecosystem as a whole (Elbasiouny et al., 2022). After treatment with Ag-NPs at a high concentration, chemical hazards on plants cause free radical damage to living tissue, resulting in DNA damage (Chowdappa and Gowda. 2013). Kushwah and Patel (2020) saw that the optimum concentration of $TiO_2$-NPs in *V. faba* ranges from 5-50 mg/L. In *Vicia faba*, silver nanoparticles also caused chromosomal aberrations (Patlolla et al., 2012).
4. Proper protection, risk assessment, testing priorities, and regulatory guidance are necessary to commercialize NMs for agricultural applications (Chen et al., 2011).
5. Plant leaves and floral parts can be coated with nanopesticides in the air. These pesticides can clog stomata and form a toxic physical barrier over the stigma, preventing tube penetration in the stigma and pollen germination. The NPs have the potential for phytotoxicity and can enter the plant's vascular tissues, preventing the movement of minerals, water, and photosynthates rate (Rico et al., 2011).
6. NPs have the potential to enter deep into the lungs of animals and humans, causing a variety of health problems such as acute or long term lung damage, asthma, and thrombosis.
7. Due to their increased transport, longer persistence, and higher reactivity, nanopesticides may contaminate water bodies and soil and become part of food webs (Pestovsky et al., 2017).

The toxicological effects of NMs are influenced by their chemical and physical properties and also depend upon plant species (Kwak et al., 2017 ; Rastogi et al., 2017). It was observed that phytotoxicity is also affected by surface modification of NMs, for example, various toxicity levels of QDs coated or capped with different materials (Rico et al., 2015 ; Singh et al., 2019). The fact that different plant species respond differently to the same NMs is yet another explanation for the apparent differences in nanotoxicity in plants (Wang et al., 2016). For instance, when lanthanide-doped upconversion NPs were applied to pumpkin plants, there was no evidence of toxicity; however, varying degrees of toxicity were observed in various plant species, including minor toxicity in tobacco (Zhu et al., 2008). Furthermore, the characteristics of the growing medium used for plants impact the NMs' phytotoxicity (Schlich and Hund-Rinke. 2015). The ability of different growth environments, such as soil or agar, to interact with NMs varies, which may alter their chemical and physical characteristics,hence phytotoxicity (Zou et al., 2016 ; Tripathi et al., 2017). For instance, pumpkin plants grown in sand and soil had distinct toxicity levels by $Fe_3O_4$-NPs (Zhu et al., 2008 ; Wang et al., 2011). Likewise, $CeO_2$-NPs toxicity was found in lettuce seeds grown in various media, including agar, sand, and potting mix (Yang et al., 2017). Additionally, NMs may act distinctively in different waters: they get highly influenced by the different types of organic matter or colloids present in fresh water and tend to agglomerate in seawater and hard water, resulting in varying degrees of phytotoxicity (Khan et al., 2019).

Nanotoxicology, a new field of toxicology, evaluates the NP's toxicity both in the lab and the field. Therefore, nanotoxicologists should collaborate with material scientists and chemists when nano-based products are to be fabricated. Toxicity assessments are essential for ensuring that newly developed nanopesticides and nanofertilizers are safe for mammals, plants, and beneficial microbes in the soil.

## 10. Future Perspective

In this review, we have highlighted many research studies aiming at nanotechnological developments in mitigating the biotic stress in plants. Pathogen detection and disease suppression have already been revolutionized by nanotechnology. Plant pathogens and microbes are increasingly being used to synthesize nanoparticles. However, plant disease management requires more research on nanotechnology's practical agricultural applications.Long-term monitoring should be subjected to assessing the impact of NPs on pathogens, as well as on human and environmental heath. Few studies assessed the long-term impacts of using NPs to control pests. For example, Mitter et al. (2017) examined BioClay, a topical NPs/RNAi delivery platform, to protect plants against viruses twenty days after its application. Similarly, Zhao et al. (2017) examined the pesticides' impacts for 48 days after applying fabricated nanoformulation. Yang et al. (2009) also tested the insecticidal potential of their formulation in stored grains for five months after application. Jenne et al. (2018) examined azadirachtin in ZnO- and chitosan-NPs against groundnut bruchid insects in a jar of stored nuts for over 180 days. When developing nanopesticides, it is important to consider the fate and safety of nanopesticides in long-term field trials, the cost of production, the optimum dose, and legislative restrictions (Parisi et al., 2015 ; Mishra et al., 2017).

Moreover, novel strategies for managing diseases are badly needed to aid in further studies on disease diagnostics, disease mitigation, and the physiology of pathogens and hosts. The plant needs smart delivery systems to absorb and transfer nanoparticles effectively to the targeted sites for precision crop protection. Nanoformulations must be preferable to avoid releasing nanoparticles into the environment. Nanotechnological methods can be used in controlled ways to create new materials that will make it easier to create analytical systems that are more sensitive, faster, and more reliable. Integrating the new techniques and tools to produce robust analytical data and risk assessment may also be the key to gaining regulatory approval. In addition, the molecular mechanism of NP-plant interaction must be the focus of future nanophytopathology. Nanophytopathology is a fascinating area of study that needs more in-depth research to make crop protection safer and better for the environment.

With nanopesticides, pests and pathogens are targeted more effectively while off-target effects are minimized. Nevertheless, it is essential to assess the potential risks of nanopesticides, including their effects on soil health and long-term environmental effects. For example, nanomaterials can be engineered to deliver antimicrobials and pesticides in controlled releases (An et al., 2022). Doing so makes it possible to apply chemicals precisely, minimizing both the chemicals used and contamination of the environment. Using nanocarriers can help direct the delivery of pesticides and antimicrobials to the affected plant tissue or infection site, increasing efficacy while reducing environmental exposure (Wang et al., 2022). In addition, plant diseases and pests can be detected and monitored using nanoscale devices, such as nanosensors and nanoprobes. A nanotechnology-based sensing platform may also provide real-time monitoring of environmental parameters, such as temperature, humidity, and soil conditions.

## Conclusions

In recent years, nanotechnology has gathered much attention and limelight in agriculture and the food industry because of its incredible potential to increase plant growth and performance, as well as to enhance resistance to stresses, whether abiotic or biotic. In this review, we have underscored the most updated and novel studies and research on the practical applications of NPs to combat biotic stresses in plants. The beneficial effects of nanomaterials on pathogen-exposed plants at the physiological, metabolic, and molecular levels are reported in studies conducted under controlled and field conditions. The effects can be regulated by various factors, such as the concentration, application method, type of NP used, and the type and intensity of plant exposure to stress. Generally, these NPs can enhance plant performance and frame a long-term strategy to mitigate the harmful effects of biotic stressors (e.g., bacteria, viruses, fungi, insects, and nematodes) in plants  and food crops. Nanotechnology has numerous applications in agriculture, medicine, food packaging, ; however, additional research is required to assess its impact on human and environmental health. Nanotechnology can come up with better solutions for agricultural applications and has the potential to revolutionize the phytotechnology used in disease management in plants. Nanotechnology has potential to play a significant role in the future, in developing and advancing multiple novel techniques for plant health improvement, by introducing novel applications in disease diagnostics and control; and further improving overall plant health by strengthening the immune system.

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